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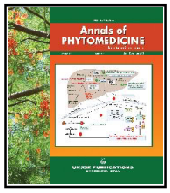

Review Article : Open Access

Management of *Candida albicans* infections by lactic acid bacteria

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Abstract

Candida albicans infections are a substantial cause of illness burden in immunocompromised patients. The opportunistic pathogen, *C. albicans* is polymorphic in nature which shows commensalism in humans, and colonizes mostly mucosal surfaces of the human body, including the respiratory, urinary, genital, gastrointestinal tracts and oropharyngeal cavity. It can cause infections which range from superficial infections such as oral thrush to serious infections like candidemia and disseminated candidiasis. These infections occur when *Candida* spp. undergo a reversible morphological transition from yeast to filamentous form. Significant efforts are focused on the mechanisms that control this transition. Antifungal drugs such as azoles, polyenes, allylamines and echinocandins are most commonly used in the treatment of *Candida* infected patients. The toxicity of antifungal medications on human cells, coupled with the increasing resistance of *Candida* infections to these drugs, highlights the necessity for the development of novel strategies to enhance the well-being of patients affected by such infections. *Lactobacillus* spp. found in the human microbiome are the natural competitors of *Candida* spp. and have the potential to control fungal growth. Lactic acid bacteria are cocci or rod-shaped, Gram-positive, non-spore forming bacteria. LAB produces lactic acid and other metabolites which suppress filamentation, a key virulence feature of *C. albicans*. This review provides a comprehensive overview of novel strategies in the prevention of *Candida* infections by highlighting the significance of LAB.

1. Introduction

It is estimated that there are around 8.7 million eukaryotic species exist in the world, and out of this staggering number, fungi account for approximately 7%, or about 611,000 species. However, only a small fraction of these fungi, about 600 species, are known to cause diseases in humans (Talapko *et al*., 2021). Despite their small numbers, these fungi can cause a wide range of diseases from mild infections of skin caused by dermatophytes and *Malassezia* species, to more serious cutaneous infections caused by *Sporotrix schenkii*, and even life-threatening systemic infections like those caused by *Cryptococcus neoformans, Aspergillus fugmigatus, Histoplasma capsulatum* and *Candida albicans* (Vaishali *et al*., 2021).

Candida is a diverse genus of fungi consisting of more than 200 species (Zeise *et al.,* 2021) among the *Candida* spp., *C. albicans* is the most common fungal pathogen of human diseases, followed by *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei* (Giri and Kindo, 2012). The threat of systemic infections from *Candida* spp. looms large, with hospitalized patients facing a crude mortality rate of more than 50% (Talapko *et al*., 2021). While *C. albicans* typically inhabits mucosal surfaces without causing harm, it can cause two major types of infection: superficial infections like oropharyngeal candidiasis (OPC), chronic mucocutaneous candidiasis (CMC), vulvovaginal candidiasis (VVC), and lethal infections such as invasive candidiasis (*e.g.,* candidemia and disseminated candidiasis) (Vaishali *et al*., 2021). Hospital-acquired bloodstream infections caused by *Candida* spp. are a significant concern for patients undergoing surgery, as well as for those with underlying immunological deficiencies and/or chemotherapeutic interventions (Wisplinghoff *et al*., 2004; Pfaller and Diekema, 2007). Patients with neutropenic cancer and organ transplants face an especially high risk of *Candida* infections (Kumar *et al*., 2012).

Candida spp. have an intriguing ability to transform from their harmless yeast form to a filamentous hyphae form, which facilitates their invasion and penetration into tissues. Many modern diagnostic technologies such as MALDI-TOF-MS, real-time PCR and DNA microarray have been developed to identify fungal infections (Kabir and Ahmad, 2013). However, the antifungal drugs currently available are limited to only a few classes of compounds, including polyenes, allylamines, azoles, fluoropyrimidines and echinocandins. Unfortunately, the widespread use of these antifungal agents has fueled the emergence of drug-resistant strains, further complicating the management of *Candida* infections. Adding to the complexity is the fact that *Candida* biofilms on catheters can cause bloodstream infections and catheter-related infections caused by *Candida* and other microbial biofilms contribute to an overwhelming 90% of hospital-acquired infections, presenting a significant cause of morbidity and mortality (Costerton, 1995).

The advent of newly discovered antifungal drugs, antibiotics, vaccines, and natural products have emerged as crucial alternatives that can be

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used either independently or in conjunction with antifungal drugs to mitigate the side effects and toxicity associated with conventional antifungal therapies (Mortale and Karrupayil, 2018). Compared to their synthetic counterparts, natural products possess several advantages, including structural diversity and a relatively low toxicity towards human cells. In healthy individuals, the growth of fungi is kept in check by the immune system and the actions of other commensal microorganisms present in the human microbiome. However, when these barriers are disrupted, *C. albicans* can begin to act as pathogen, resulting in various infections. This article will provide an overview of *Candida* infections, the drugs used to treat candidiasis, drug resistance, certain preventive measures and role of lactic acid bacteria in combating *C. albicans* infections.

2. Infections caused by *Candida* spp.

C. albicans is an opportunistic pathogen that can cause various types of infections under certain circumstances, such as changes in environmental pH and exposure to stress conditions. Candidiasis can affect both healthy and immunocompromised individuals (Liu *et al*., 2006), and it can be categorized as superficial mucosal candidiasis and invasive candidiasis. OPC occurs in high-risk patients, such as diabetic patients, dental wearers, patients treated with broad-spectrum antibiotics, and those infected with HIV (Vaishali *et al*., 2021). In fact, approximately 84% of individuals infected with HIV are asymptomatically colonized by *Candida* spp. in the oral cavity (Sangeorzan *et al*., 1994), which is considered an important marker for the onset of AIDS. CMC is caused by a heterogeneous group of primary immune deficiencies that are not able to clear fungal infections, resulting in recurrent infections of the skin and mucosal membranes with *C. albicans* (Lilic, 2002). Patients with chronic and recurring *C. albicans* infections of the skin, nails, and mucous membranes are said to have chronic mucocutaneous candidiasis, a complicated condition. Patients with CMC can be divided into a number of subgroups based on the distribution and severity of the *Candida* infections, as well as autoimmune illnesses, endocrino pathies, thymomas, and interstitial keratitis as possible concomitant conditions. In patients with persistent mucocutaneous candidiasis, a number of additional diseases may also coexist. Alopecia totalis, vitiligo, tooth enamel dysplasia, various infectious disorders, and endocrinopathies are a few of these. The most common type of mucosal candidiasis is VVC, affecting up to 75% of women of child-bearing age (Cassone *et al*., 2007). VVC is characterized by itching, irritation, persistent genital pain, and vaginal discharge. There are two types of VVC: uncomplicated and complicated VVC. Complicated VVC is defined as a recurrent disease in which an individual experience more than four episodes of symptomatic VVC within one year (Sobel *et al*., 2004). Primary immunodeficiencies can also be a cause of mucosal candidiasis. Unlike superficial candidiasis, invasive candidiasis is more deadly categorized as disseminated candidiasis and candidemia. Disseminated candidiasis caused by *Candida* is a severe deep-seated organ infection that affects the liver and spleen. The clinical spectrum of disseminated candidiasis varies from fever to severe sepsis with multi-organ failure (Gurey *et al*., 2009). Pathogens can enter the intravascular compartment through an indwelling intravascular catheter site or from the gastrointestinal tract or skin. Candidemia accounts for 15% of bloodstream infections in hospitals, with *Candida* spp. being the main causative agents in 60% of systemic fungal infections (Barchiesi *et al*., 2016).

3. Pathogenicity mechanisms

C. albicans is a species which presents a high degree of flexibility and is able to infect a diverse host niche because of its great array of fitness attributes and virulence factors as described in Figure 1. Fitness attributes like being able to rapidly adapt in an extremely wide range of environments regarding to the nutrient's availability, variations in temperature, pH, osmolarity and amount of oxygen available (Paramythiotou *et al*., 2014). Virulence factors such as morphological transition between yeast and hyphal form, adhesins and invasins expression on the cell surface, formation of biofilms, hydrolytic enzyme secretion, thigmotropism and contact sensing (Mayer *et al*., 2013).

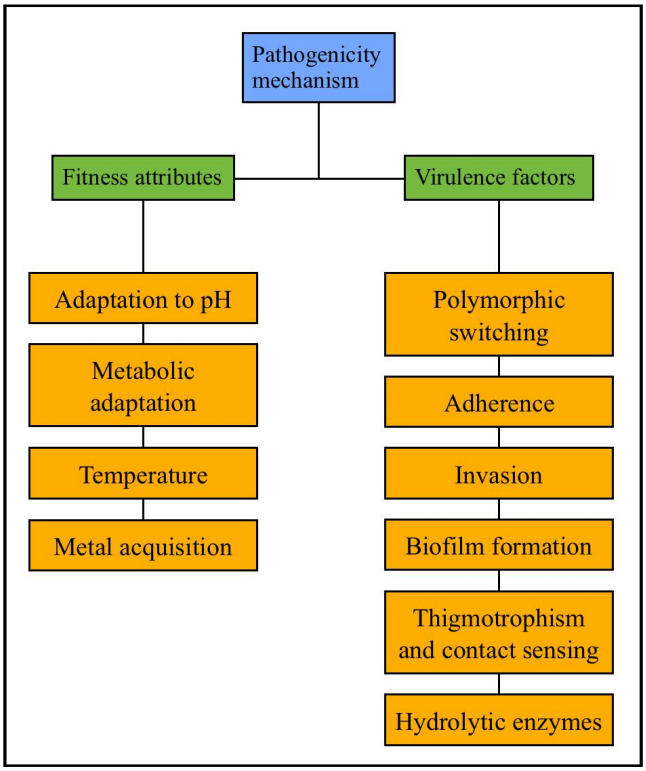

Figure 1: Different types of pathogenicity mechanism.

3.1 Fitness attributes of *C. albicans*

3.1.1 Adaptation to pH

C. albicans is a common opportunistic fungal pathogen that can cause a range of infections in humans. In order to survive in the host, *C. albicans* must be able to adapt to a dynamic range of pH environments. For example, the pH of human blood is slightly alkaline (pH 7.4), the digestive tract ranges from very acidic (pH 2) to alkaline (pH 8), and the vaginal pH is around pH 4 (Davis, 2009). Therefore, *C. albicans* needs to be able to handle this dynamic pH environment in order to survive. The ability of *C. albicans* to adapt to pH changes is crucial for its survival in different host niches. When the pH is neutral to alkaline and nutrient acquisition is impaired, *C. albicans* experiences acute stress (Mayer *et al*., 2013). *C. albicans* has evolved a range of strategies to thrive and survive in different pH environments, including the activation of signal transduction pathways, secretion of pH-modulating enzymes, and expression of pH-responsive genes (Mayer *et al*., 2013). These features collectively

underscore the importance of understanding the pH-dependent mechanisms underlying adaptation and virulence of *C. albicans*, and the role they play in this fungal pathogen's successful colonization and infection of human hosts. To adapt to changing pH levels, the fungus uses cell wall β-glycosidases Phr1 and Phr2. PHR1 is essential for systemic infections and is expressed at neutral to alkaline pH, while PHR2 is mainly expressed at acidic pH and is required for vaginal infections (Miao *et al*., 2020).

The Rim101 signal transduction pathway is responsible for sensing pH levels in *C. albicans*. This pathway involves the plasma membrane receptors Rim2 and Dfg16, which measure the environmental pH. When these receptors are activated, a signaling cascade is induced, leading to the activation of the major pH-responsive transcription factor Rim101. Rim101 enters the nucleus and coordinates pH-dependent responses, allowing *C. albicans* to adapt to changing pH environments (Davis, 2009). Studies have shown that pH sensing by the Rim101 pathway is critical for *C. albicans* virulence. Mutant forms of *C. albicans* with a defect in the Rim101 pathway had reduced virulence in both a mouse model of oropharyngeal candidiasis and a systemic mouse model of hematogenously disseminated candidiasis (Davis *et al*., 2000). This highlights the importance of pH sensing in the pathogenicity of *C. albicans*, and emphasizes the significance of Rim101 pathway in the survival and virulence of *C. albicans*. In addition to its pH adaptability, *C. albicans* has the capacity to regulate extracellular pH under conditions of nutrient scarcity. This enables the fungus to alkalinize its surrounding environment, triggering the autoinduction of hyphae formation, a critical virulence factor of *C. albicans* (Vylkova *et al*., 2011; Mayer *et al*., 2012). The ability of fungus to sense, adapt, and modulate extracellular pH levels is thus instrumental in its ability to coexist as a commensal organism, while also allowing it to manifest as a fungal pathogen in humans.

3.1.2 Metabolic adaptation

Metabolic flexibility is crucial for pathogenic fungi during infection in different host niches because nutrition is a prerequisite for the survival and growth of all living organisms. Metabolic adaptation enables fungi to assimilate alternative nutrients in a changing environment (Mayer *et al*., 2013). *C. albicans* typically resides in the gastrointestinal microbiome of healthy individuals, where it competes with other microbes, despite the abundance of nutrients in the gut. However, in susceptible individuals with disseminated candidiasis, *C. albicans* can gain access to the bloodstream, which is rich in glucose, the preferred nutrient source for most fungi (Brock, 2009). Phagocytic cells such as neutrophils and macrophages can efficiently phagocytose *C. albicans*, creating a hostile environment with restricted nutrient availability due to the production of reactive intermediates such as reactive oxygen species, reactive nitrogen species and antimicrobial peptides (Frohner *et al*., 2009). To survive in this environment, *C. albicans* switches from glycolysis to gluconeogenesis and activates a starvation response through glyoxylate cycle activation. Inside macrophages, amino acids and lipids serve as nutrient sources for *C. albicans* (Lorenz *et al*., 2004). In addition to metabolic flexibility, *C. albicans* have generated ways to escape from macrophages by inducing hyphal formation and inhibiting the antimicrobial effectors' production. Hyphae formed inside phagocytic cells can help the fungus pierce through the host immune system by using mechanical forces (Lorenz *et al*., 2004; Ghosh *et al*., 2009). The ability of *C. albicans* to respond rapidly and dynamically to micro-environmental nutrient availability makes *C. albicans* a more virulent fungal pathogen.

3.1.3 Temperature adaptation

High temperature causes the unfolding and aggregation of non-specific proteins, creating a stressful condition that ultimately leads to cell death. To prevent thermal stress, heat shock proteins (Hsps) are produced by fungal cells, which act as chaperones that prevent protein unfolding and aggregation and finally stabilize them (Mayer *et al*., 2013). The transcription factor, heat shock factor 1 (Hsf1) regulates the expression of Hsps. Mutants that are unable to activate Hsf1 show reduced virulence in a mouse model of systemic candidiasis, suggesting the essentiality of Hsf1 for *C. albicans* pathogenicity and viability in the host (Mayer *et al*., 2013). In *C. albicans*, six Hsps have been identified: and out of those the Hsp60 was found to be necessary to tolerate thermal stress (Leach *et al*., 2011). Besides the above-mentioned Hsps, six small heat shock proteins (sHsps) have also been identified in *C. albicans*. sHsps are chaperones with a low molecular weight that prevent protein aggregation (Narberhaus, 2002). During thermal or other kinds of stress, sHsps are expressed by the cell, and they bind aggregated proteins by transitioning from oligomeric form to multimeric state (Eyles and Gierasch, 2010). Clint proteins in these chaperone-aggregated complexes are kept ready for disaggregation and refolding by other major Hsps. The Hsp12 is expressed in response to different stresses, such as oxidative stress and thermal stress. A *C. albicans* mutant with both HSP12 genes deleted did not cause virulence in a Drosophila infection model (Fu *et al*., 2012). The investigations on Hsp21 have shown its essential role in the regulation of intracellular trehalose levels. *C. albicans* mutants with HSP21 deletions exhibit enhanced oxidative stress sensitivity, impaired thermotolerance, and strongly attenuated pathogenicity in a mouse model of systemic candidiasis (Mayer *et al*., 2013).

3.1.4 Metal acquisition

Trace metals are essential for the survival and growth of all living organisms, including bacteria, fungi, plants, and animals. Several important metals such as zinc, manganese, iron, and copper are required for the proper functioning of numerous proteins and enzymes. Pathogens and their hosts have developed intricate mechanisms to restrict or acquire access to these metals (Hood and Skaar, 2012). Among the transition metals investigated in pathogenesis, iron has been the most widely studied. *C. albicans*, for instance, acquires iron through various systems, such as the heme-iron uptake system, siderophore system, and reductive system (Almeida *et al*., 2009). The heme-iron uptake system enables iron acquisition *via* hemeproteins and hemoglobin, and it is mediated by members of the heme-receptors gene family, including CSA1, CSA2, RBT5, RBT51, and PGA7. Though the roles of Csa1, Csa2, Rbt51, and Pga7 in *Candida* virulence have yet to be investigated, the rbt5Ä/Ä mutant shows normal virulence in mice, which might be due to functional redundancy (Weissman and Kornitzer, 2004; Almeida *et al*., 2009). Siderophores, on the other hand, are not synthesized by *C. albicans* on their own, but they can use siderophores generated by other microorganisms (also known as xeno-siderophores) to steal iron. *C. albicans* uptake system is facilitated by Sit1, the only siderophore transporter in *C. albicans*. A sit1Ä/Ä mutant exhibits normal virulence in a mouse model of disseminated candidiasis.

However, the mutant has a severely impaired capacity to damage human keratinocyte tissue in *ex vivo* condition (Heymann *et al*., 2002).

Iron acquisition *via* the reductive system is mediated from host transferrin, ferritin, or the environment. Als3 (adhesin and invasin) is the receptor for ferritin. Iron acquisition with the help of Als3 from host ferritin contributes to iron acquisition depending on the infection stage (Mayer *et al*., 2013). Deletion of the ALS3 gene reduces the capacity to damage in vitro oral epithelial host cells, although an als3Ä/Ä mutant shows normal virulence in a mouse model of disseminated candidiasis (Cleary *et al*., 2011). After iron, zinc is the second most abundant metal in most living organisms. The zinc-binding protein Pra1 (pH-regulated antigen 1) secreted by fungi acts as a zincophore when it binds to extracellular zinc and re-associates with fungal cells. This process is similar to the siderophore-mediated acquisition of iron. Pra1 re-association is mediated by Zrt1, a zinc transporter. Deletion of the PRA1 gene reduces capacity of *C. albicans* to damage endothelial cells in vitro when exogenous zinc is absent (Soloviev *et al*., 2011). This suggests that zinc acquisition plays a critical role during certain stages of *Candida* infections. The mechanisms of copper and manganese acquisition by *C. albicans* are still poorly understood but these metals are also essential for fungal growth.

3.2 Virulence factors

3.2.1 Polymorphic switching

C. albicans exhibits various morphologies, including yeast (ovoid-shaped budding), pseudohyphae (ellipsoid-shaped cells with septal constrictions), true hyphae (parallel-walled filaments), chlamydospores (thick-walled spore-like structures), and white/opaque cells (formed during the transition stage) (Berman and Sudbery, 2002). Each morphology type displays different pathogenicity levels, and the transition between yeast and hyphae is known as dimorphism. The morphology of *C. albicans* is affected by a wide range of environmental factors at low pH (<6), *C. albicans* grows in the yeast form, while at high pH (>7) it grows in hyphal form. Other factors, such as starvation, physiological temperature, carbon dioxide, and the presence of serum or N-acetyl glucosamine, promote hyphae formation. Quorum sensing, a microbial communication mechanism, also regulates the morphogenesis of *C. albicans* (Hornby *et al*., 2001). As a result of yeast growth is favored at high cell densities (>10^7 cells ml^{-1}), while hyphal growth is promoted at low cell densities (<10^7 cells ml^{-1}). Farnesol, tyrosol, and dodecanol are the main quorum sensing molecules for *C. albicans* (Mayer *et al*., 2013).

3.2.2 Adherence

Adhesins are a critical class of proteins in *C. albicans* that enable the organism to adhere to a variety of surfaces, including other microorganisms, host cells, and abiotic surfaces (Garcia *et al*., 2011). The agglutinin-like sequence (ALS) family of proteins comprises eight members and is the most extensively studied adhesins in *C. albicans*. Among these proteins, Als-3, an adhesin associated with hyphal growth, plays a pivotal role in promoting adhesion (Murciano *et al*., 2012). During *in vitro* infection of oral epithelial cells and *in vivo* vaginal infections, there is an upregulation of the ALS3 gene expression (Wachtler *et al*., 2011).

3.2.3 Invasion

C. albicans utilizes two primary mechanisms to invade host cells: active penetration and induced endocytosis (Mayer *et al*., 2013). Active penetration is a fungal-driven process that requires viable *C. albicans* hyphae (Dalle *et al*., 2010; Wachtler *et al*., 2011), while induced endocytosis is a process whereby the fungus expresses specialized proteins, known as invasins, that mediate binding to host ligands (such as E-cadherin on epithelial cells and N-cadherin on endothelial cells), and thereby trigger the engulfment of fungal cells into the host cells. Interestingly, the viability of fungal cells does not matter for induced endocytosis, as this process is passive and can even uptake killed or dead hyphae (Dalle *et al*., 2010). Of the invasions, Als-3 (which also acts as an adhesin) and Ssa1 are the most notable (Phan *et al*, 2007; Sun *et al*., 2010). Ssa1 is a member of the heat shock protein 70 (Hsp70) family and is expressed on the cell surface. Both Als-3 and Ssa1 induce endocytosis by binding to host E-cadherin. However, in *C. albicans*, macropinocytosis also plays a role in induced endocytosis (Dalle *et al*., 2010).

3.2.4 Biofilm formation

C. albicans is capable of forming biofilms on both biotic (*e.g.*, mucosal cell surfaces) and abiotic (*e.g.*, catheters and dentures) surfaces, which is a key virulence factor in *C. albicans* infections (Taff *et al*., 2012). Biofilm formation is a complex process that involves a series of events such as adherence of yeast cells to the substrate, yeast cell proliferation, formation of hyphal cells, accumulation of extracellular matrix material, and finally, dispersion of yeast cells from the biofilm complex, all of which occur in a sequential manner (Kanwar *et al*., 2019). In comparison to planktonic cells, mature biofilms exhibit greater resistance to host immune factors and antifungal agents (Finkel and Mitchell, 2011; Fanning and Mitchell, 2012). The factors responsible for this increased resistance include the complex biofilm architecture, biofilm matrix, metabolic plasticity, and increased expression of drug efflux pumps (Fanning and Mitchell, 2012). The Hsp 90 has been identified as the major heat shock protein that regulates the dispersion of *C. albicans* biofilms and is also required for biofilm antifungal drug resistance (Robbins *et al*., 2011).

Biofilm formation in *C. albicans* is regulated by different transcription factors such as Tec1, Bcr1, and Efg1 (Fanning and Mitchell, 2012). Additional factors control the production of extracellular matrix, such as the zinc-responsive transcription factor Zap1, which negatively regulates β-1,3 glucan, a major component of the biofilm matrix. Meanwhile, the positive regulators of β-1,3 glucan production are glucoamylases (Gca1 and Gca2), glucan transferases (Bgl2 and Phr1), and exoglucanase (Xog1) (Nobile *et al*., 2009). β-1,3 glucan present in the extracellular matrix protects *C. albicans* from host immune factors and antifungal agents.

3.2.5 Thigmotropism and contact sensing

The sensing of contact is a critical environmental cue that triggers the formation of hyphae and biofilms in *C. albicans*. When these yeast cells come into contact with surfaces, they undergo a transition to hyphal growth, which can enable them to invade the substratum of certain surfaces such as agar and mucosa (Kumamoto, 2008). In fact, biofilm formation is often induced by the contact with solid surfaces. This phenomenon, known as thigmotropism, is the directional growth of hyphae that occurs on surfaces with specific topologies, such as the presence of ridges on the surface (Mayer *et al*., 2013).

Thigmotropism is regulated by the uptake of extracellular calcium through calcium channels Mid1 and Cch1. Brand *et al*. (2008) provide evidence of the requirement of *C. albicans* thigmotropism to fully damage epithelial cells and to have normal virulence in mice. Therefore, the accurate sensing and response to both biotic and abiotic surfaces is crucial for the pathogenicity of *Candida*.

3.2.6 Hydrolytic enzymes

C. albicans hyphae play an important role in the pathogenicity of this fungus, as they can secrete hydrolases that facilitate the active penetration into host cells after adhering to host cell surfaces and undergoing hyphal growth (Wachtler *et al*., 2012). This process also increases the efficiency of nutrient acquisition from the extracellular environment. Three classes of hydrolases are mainly expressed by *C. albicans*: proteases, lipases, and phospholipases. The secreted aspartic proteases (Saps) comprise ten members, Sap1-10, and while Sap9 and Sap10 remain bound to the cell surface, Sap1-Sap8 are secreted into the surrounding medium. Among these, Sap1-Sap3 are required *in vitro* to damage the reconstituted human epithelium and for pathogenicity in a mouse model of systemic infection, although the exact contribution of Saps to *C. albican*s pathogenicity remains controversial (Albrecht *et al*., 2006).

The family of lipases also consists of ten members (Lip1-10) and a lip8Δ/Δ mutant has been shown to have reduced virulence in a mouse model of systemic infection, indicating the involvement of these extracellular hydrolases in the pathogenicity of *C. albicans* (Gacser *et al*., 2007). The phospholipase family is composed of four different classes: A, B, C and D, but only five extracellular members of class B (PLB1-5) have been shown to contribute to pathogenicity by disrupting host plasma membranes. Both plb1Δ/Δ and plb5Δ/Δ mutants displayed weakened virulence in a mouse model of systemic infection (Mavor *et al*., 2005; Theiss *et al*., 2006). These findings suggest that hydrolases play an important role in the pathogenicity of *C. albicans* and highlight the need for further investigation of the mechanisms involved in their secretion and function during infection.

4. Host defense against *Candida* infection

Host defense against *Candida* infection mainly relies on entire mucosal and skin barriers, and appropriate fungal recognition. These actions trigger the protective innate and adaptive defense mechanisms against fungal pathogens (Netea *et al*., 2008). Skin and mucosa are the first line of defense, offering a mechanical barrier and microbial antagonism. Protective innate and adaptive immune mechanisms activate only after the first line of defense has failed and critically depend on adequate recognition of fungal pathogens. *Candida* recognition is mediated by pattern recognition receptors (PRRs) that bind with pathogen-associated molecular patterns (PAMPs). Components of fungal cell walls, such as heavily glycosylated proteins by O-linked and N-linked mannosylation as well as β-(1,3)-glucan covalently linked with β-(1,6)-glucan (Iorio *et al*., 2008) and chitin, are the most studied and known PAMPs of *Candida*. All of these cell wall components act as ligands for one or more PRRs. The two main classes of PRRs in *Candida* recognition are toll-like receptors (TLRs), such as TLR2, TLR4, and C-type lectin receptors, such as dectin-1, dectin-2, mincle, and mannose receptors (Wells *et al*., 2008; Robinson *et al*., 2009). At the site of infection, the interaction between PRRs expressed by different types of cells present determines the initiated response of *Candida*.

Monocytes, macrophages, and polymorphonuclear cells (PMNs) are necessary for the main innate effector responses, such as induction of ROS and *Candida* phagocytosis, that can damage and eliminate fungus. For proper activation of PMNs, pro-inflammatory cytokines like interleukin (IL)-1β and tumor necrosis factor α (TNFα) are crucially required. TNF α is essential for the anti-*Candida* defense of the host *via* phagocytosis and neutrophil recruitment. TNFα deficiency resulted in higher mortality during experimental disseminated candidiasis (Netea *et al*., 2004). Moreover, CD4 T lymphocytes produce IFNγ which induces *Candida*-specific immunoglobulin production and NO production by macrophages, also playing an important role in the stimulation of PMNs' antifungal activity. The finding that knockout mice defective in IFNγ are very susceptible to *C. albicans* infection has highlighted the crucial role of endogenous IFNγ in the resistance against systemic candidiasis. Disseminated candidiasis is also more prevalent in mice lacking the cytokine IL-18, which is essential for the induction of IFNγ (Netea *et al*., 2003). Hence, faults that prevent *Candida* from being killed by phagocytosis and defects that cause IFNγ production appear to be related to the pathophysiology of invasive candidiasis.

Epithelial cells are a significant source of pro-inflammatory cytokines in the mucosa, and they are known to play a central role in protecting against fungal pathogens (Van De Veerdonk *et al*., 2010). Several studies have advocated the involvement of epithelial cells in the production of interleukin-8 (IL-8) and granulocyte-macrophage colony-stimulating factor (GM-CSF) in response to *Candida* spp. infection. Oral epithelial cells have also been found to up-regulate the antifungal activity of neutrophils *in vitro*, and this effect is believed to be partially dependent on IL-1a. Neutrophils may also up-regulate Toll-like receptor 4 (TLR4) expression in response to *C. albicans*-infected human oral epithelium, which has been directly linked to protection against fungal invasion of the epithelium (Dongari-Bagtzoglou *et al*., 2005).

Th17 (IL-17/IL-22-producing) cells and CD4+ T helper (Th)1 (IFNγ-producing) cells are responsible for stimulating the adaptive immune responses necessary for antifungal defense. Antigen presentation in the presence of the cytokine IL-12 triggers the Th1 response that is specific to *Candida*. On the other hand, the presence of IL-1 and IL-23 induces and maintains Th17 responses. Furthermore, oral candidiasis often occurs in people with HIV who have low CD4 levels, emphasizing the crucial role T helper cells play in the host's mucosal anti-*Candida* response. CD8+T cells have also been found to be critical for anti-*Candida* host defense in the absence of CD4+T cells.

Overall, these findings highlight the complex interplay between epithelial cells and various immune cells in the host's anti-*Candida* defense mechanisms. Further studies are underway to elucidate the precise mechanisms underlying the role of epithelial cells in the immune response to *Candida* infection, with the ultimate goal of developing targeted therapies for oral candidiasis.

5. Medication available to combat fungal pathogens

Invasive fungal infections are responsible for 1.7 million deaths annually worldwide (Vitiello *et al*., 2023) predominantly affecting AIDS patients, immunocompromised individuals, and organ transplant recipients. Among the most prevalent types of invasive fungal

infections are candidiasis, aspergillosis, and mucormycosis, with an annual incidence of over 750,000, 300,000, and 10,000 cases, respectively, (Houst *et al*., 2020). These infections are associated with high mortality rates, highlighting the urgent need for effective treatment options. Fungal infections have been documented for centuries, but it was not until the 1930s that the first antifungal drug, griseofulvin, was isolated from the metabolic products of *Pencillium griseofulvum*. However, its efficacy in treating fungal infections was not established until 1958, and it was not widely used for clinical purposes until then. Subsequently, more effective antifungal drugs, such as polyene and amphotericin B, were introduced in 1960 and have been considered the best antifungal drugs to date (Kabir and Ahmad, 2013). Since then, a large number of antifungal drugs have been developed and utilized in the treatment of fungal infections and invasive, life-threatening fungal diseases. These antifungal drugs kill the fungal cells by targeting different parts of fungal cell as described in Figure 2.

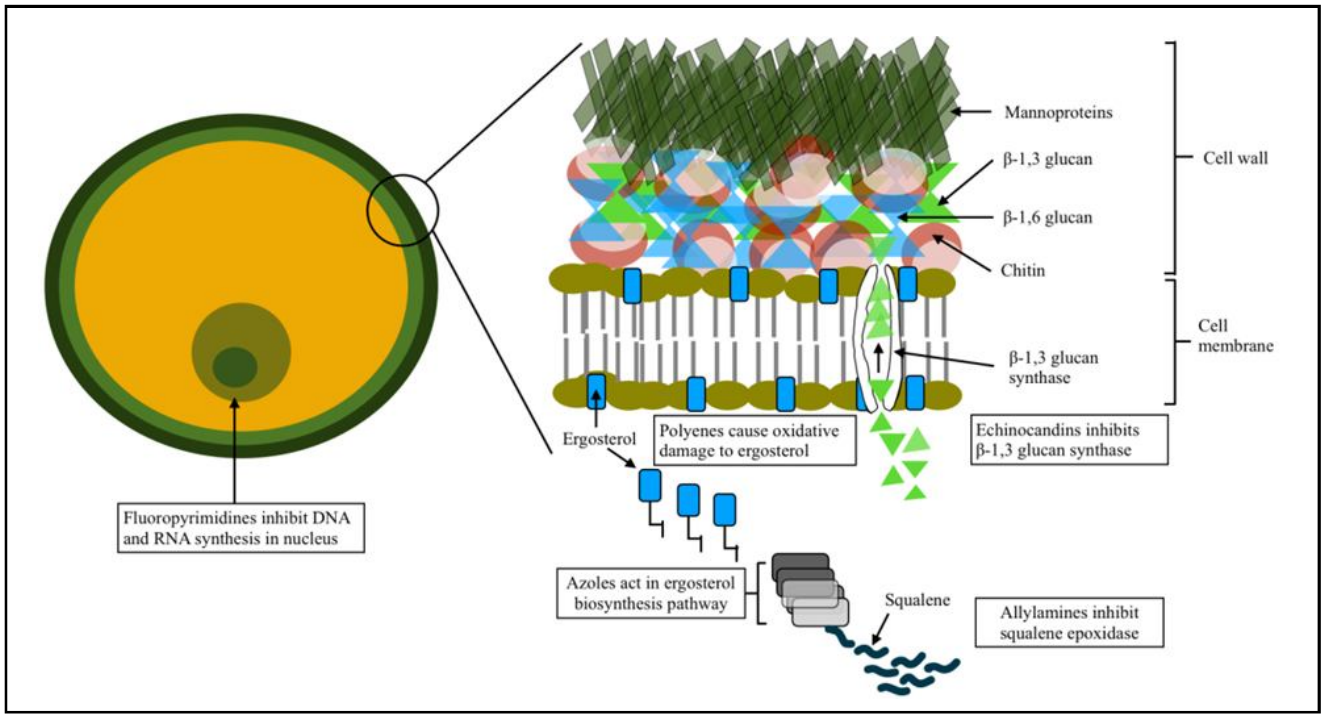

Figure 2: Different targets of antifungal drugs.

5.1 Azoles

Azoles are a widely used class of antifungal drugs that are effective against both mucosal and systemic infections caused by *C. albicans.* The first azole derivatives were introduced in the 1960s and have since expanded in number (Xu *et al*., 2011). Azole drugs are classified into imidazoles and triazoles, depending on the presence of two or three nitrogens in the five-membered azole ring. They work by inhibiting the ergosterol biosynthetic pathway, which is essential for maintaining the asymmetry and fluidity of the fungal cell membrane, and the overall integrity of the cell wall. Ergosterol is the primary component of the fungal cell wall and acts as a bioregulator (Cerqueira *et al*., 2021).

Azoles decrease the level of ergosterol in the cell membrane by inhibiting the cytochrome P450-dependent enzyme lanosterol 14-α-demethylase which is encoded by the ERG11 gene. Fungal lanosterol demethylation is a two-step process that involves the reduced form of both oxygen and nicotinamide dinucleotide phosphate (NADPH). The nitrogen from the triazole ring binds to heme iron, thereby preventing the oxidation of the methyl group (Sachin *et al*., 2018). The result of this inhibition is the accumulation of toxic 14-α-methylsterols and depletion of ergosterol, leading to a fungistatic effect.

Moreover, the altered cell membrane structure due to azoles results in several other responses, such as inhibition of hyphal development, inactivation of vacuolar ATPases (V-ATPase), and changes in oxidative and nitrosative stresses. These effects have been observed in various studies and suggest that azoles may have additional mechanisms of action beyond their antifungal activity (Bhagat *et al*., 2020). In summary, azoles are an important class of antifungal drugs that target the ergosterol biosynthetic pathway. The altered cell membrane structure due to azoles also leads to several other responses that may contribute to their efficacy.

5.2 Echinocandins

This is a class of lipoproteins that have antifungal activity against *Candida* both *in vitro* and *in vivo*, discovered in the 1970s. These are fermentation products of various microorganisms and modified analogues of pneumocandins (Houst *et al*., 2020). Anidulafungin, caspofungin, and micafungin are some of the commonly used echinocandins (Toth *et al*., 2020). Anidulafungin is derived from echinocandin B0 (*Aspergillus nidulans*), caspofungin from pneumocandin B0 (*Glarea lozoyensis*), and micafungin from pneumocandin A0 (*Coleophoma empetri)* (Houst *et al*., 2020). Echinocandins are cyclic hexapeptides with specific lipophilic N-acetylated side chains, and their physicochemical properties are determined by their core. These are specific noncompetitive inhibitors

of the enzyme β-(1,3)-glucan synthase (responsible for the synthesis of β-glucan). The enzyme β-(1,3)-glucan synthase is composed of intracellular regulatory Rho1 units and transmembrane catalytic Fks, and due to inhibition, it is unable to convert uridine diphosphate glucose to β-d-glucan, making the fungal cell wall highly permeable (Houst *et al*., 2020). Anidulafungin is a semisynthetic echinocandin that is more effective against systemic candidiasis caused by *C. albicans* compared to the commonly used fluconazole. It also cleanses blood infections faster, thus having a more effective global response (Van Daele *et al*., 2019).

5.3 Fluorinated pyrimidine analog

5-Fluorocytosine (5-FC) was initially discovered as an anticancer drug in 1957, but later in 1968, it was tested for the treatment of fungal infections, particularly candidiasis and cryptococcosis, as its efficacy in cancer treatment was not proven. Although 5-fluorocytosine itself does not have antifungal activity, one of its metabolites, 5-fluorouracil (5-FU) was found to be toxic against fungal cells (Van Daele *et al*., 2019). 5-FC is a synthetic analogue of cytosine, which is taken up by cytosine permease into fungal cells after administration and deaminated into 5-FU by cytosine deaminase. Subsequently, 5-fluorouracil is converted into 5-fluorouridine triphosphate, which is incorporated into fungal RNA and inhibits protein synthesis. Uridine monophosphate pyrophosphorylase can also convert 5-fluorouracil into 5-fluorodeoxyuridine, which inhibits the primary thymidine source thymidylate synthetase in DNA biosynthesis due to the enzyme's inability to remove the fluorine atom (Houst *et al*., 2020). However, 5-FU is toxic to mammalian cells, which is why it is not administered directly to candidiasis patients. Instead, 5-FC is used as it is safer compared to 5-FU. Upon entering *Candida* cells through cytosine permease, 5-FC is rapidly converted into 5-FU, which exerts toxic effects on fungal cells (Van Daele *et al*., 2019).

5.4 Polyenes

Fungicidal antibiotics produced by *Streptomyces* spp. have a broader spectrum than other antifungal drugs (Nguyen *et al*., 2021). Polyenes, including amphotericin B (AMB), natamycin, and nystatin, are commonly used drugs that bind specifically to ergosterol, which results in damage to the cell membrane and ultimately, cell death. AMB is a cyclic heptane produced by the Gram-positive bacterium *Streptomyces nodosus*. It has a direct intermolecular interaction with ergosterol, but it also interacts with cholesterol, a component of mammalian cell membranes (Matsumori *et al*., 2009). Despite having a higher affinity towards ergosterol-containing cell membranes than cholesterol-containing or sterol-free cell membranes, AMB also interacts with mammalian cell membranes, which can lead to toxicity (Wu *et al*., 2019).

AMB has two mechanisms of action. The first mechanism occurs after several AMB molecules incorporate into the fungal cell membrane's lipid bilayer, forming membrane-spanning ion channels that cause disruptions in the plasma membrane, leading to increased membrane permeability. As a result, essential components leak out, ultimately causing death of the fungal pathogens (Al-Dhaheri and Douglas, 2010). In the second mechanism, AMB induces the accumulation of reactive oxygen species, which leads to DNA, protein, membrane, and mitochondrial damage in fungal cells. Polyenes showcase fungicidal activity by causing oxidative damage to fungal cells. While AMB has a broad spectrum against many fungal pathogens, it also has substantial high toxic effects on human cells, which can lead to renal failure in patients (Mouri *et al*., 2008).

Table 1: Properties of some of the commonly used antifungal drugs

S. No.	Antifungal drug	Mode of action	Side effects	Resistance mechanism	References
1	Azole	Inhibit lanosterol 14 α-de-methylase (a product of ERG 11 gene) in ergosterol biosynthesis	Hepatotoxicity	Alteration of specific steps in the ergosterol biosynthetic pathway and by drug efflux which occur due to decrease in Erg11 protein by mutation up-regulation of multidrug transporter genes.	(Sachin *et al*., 2018; Cerqueira *et al*., 2021)
2	Echinocandins	Inhibit β-(1,3)-glucan synthase (a product of GSC1 gene) in cell wall biosynthesis	Tachycardia, hypotension, or thrombophlebitis	By altering the echinocandins affinity for β-1,3 glucan synthase.	(Van Daele *et al*., 2019; Toth *et al*., 2020)
3	Fluorinated Pyrimidine Analog	Misincorporation of 5-flouorouracil during DNA and RNA synthesis	Hepatotoxicity, myelotoxicity, and gastrointes-tinal problems	Deregulation of pyrimidine biosynthetic pathway, due to defects in cytosine deaminase and lack of essential enzyme for 5-FC metabolism.	(Van Daele *et al*., 2019)
4	Polyenes	Increase cell membrane permeability and oxidative damage to ergosterol	infusion and dose-related toxicity	Alteration of specific steps in biosynthetic pathway due to decrease or absence in binding ergosterol content.	(Wu *et al*., 2019; Nguyen *et al*., 2021)
5	Allylamines	Inhibit squalene epoxidase (a product of ERG1 gene) in ergosterol biosynthesis	Hypersensitivity or allergic reactions and increased risk of both local and systemic toxicity	Alteration in the ERG1 gene.	(Kabir and Ahmad, 2013; Alghaith *et al*., 2021)

5.5 Allylamines

Non-competitive inhibitors of squalene epoxidase, such as allylamines, are effective against numerous fungal pathogens, including *Candida* strains that are resistant to azole drugs. Commonly used allylamines include naftifine and terbinafine. These inhibitors target the gene ERG1, which encodes the squalene epoxidase enzyme

that is critical for ergosterol biosynthesis (Van Daele *et al*., 2019). Unlike azoles, allylamines do not deplete ergosterol levels in fungal cells. Instead, they cause the accumulation of squalene, which disrupts plasma membrane formation and organization, ultimately resulting in increased membrane permeability and cell death.

Naftifine, in particular, has been shown to be highly effective in treating fungal infections and relieving associated symptoms. Additionally, naftifine has been found to possess anti-inflammatory properties, including the reduction of superoxide production and polymorphonuclear leukocyte chemotaxis (Alghaith *et al*., 2021). Therefore, it may offer advantages over other antifungal agents, particularly for the treatment of infections that are accompanied by inflammation. Various antifungal drugs with their mode of action, side effects caused by them and resistance mechanisms acquired by fungal against these antifungal drugs have been presented in Table 1.

6. Antifungal drug resistance

Overuse of antifungal drugs increases the opportunistic fungal pathogen resistance. The World Health Organization (WHO) has identified such types of antimicrobial resistance as the dominant threat of 2019 (Houst *et al*., 2020). Antifungal drug resistance is an evolutionary process which is based on the natural selection of organisms resulting in their enhanced ability to survive and grow in the presence of drugs. This process is ubiquitous in nature and microbes evolve different strategies to combat the action of antifungal drugs (Rani and Azmi, 2019). Antifungal drug resistance results from multiple factors and emerges by a series of molecular mechanisms. Naturally, intrinsic resistance has been found in some fungal species like *C. krusei*, *C. glabrata*, and *Aspergillus* species are fluconazole-resistant but acquired resistance is a consequence of widespread prophylaxis, long-term therapies, or antifungals use in agriculture, especially in the case of triazoles. In addition, secondary resistance may occur after vertical and horizontal transmission in both animals and humans (Houst *et al*., 2020).

The *Candida* infections are treated with various antifungal drugs, but drug resistance poses a serious problem to individual patient health and creates difficulties in managing healthcare systems. Factors such as the efflux of antifungal drugs from fungal cells, modifications of target sites due to point mutations in genes, modulation of transcription factors, and key enzyme modifications in biosynthetic pathways play a crucial role in drug resistance (Cannon *et al*., 2009; Morschhauser, 2010). The two main mechanisms that result in these modifications are efflux pumps and mutations in target sites.

6.1 Efflux pumps

Drug resistance in *Candida* spp. is primarily caused by efflux pumps, which are transmembrane proteins that transport various substrates across membranes using different energy sources. The two major classes of efflux pumps are ATP binding cassette (ABC) pumps, which use ATP as their energy source, and major facilitator superfamily (MFS) pumps, which use proton motive force across the plasma membrane (Kiran *et al*., 2021). Among the ABC transporters, Cdr1p and Cdr2p, and among the MFS efflux pumps, Mdrp1, have been well studied and are known to play a significant role in antifungal drug resistance in *C. albicans* (Prasad and Goffeau, 2012). The structure-function analysis of Cdr1p and Cdr2p reveals that these transporters have two distinct domains, the transmembrane domains (TMDs) and nucleotide binding domain (NBDs), which produce inward-facing cavities for drug binding. These cavities can be accessed from either the cytoplasm or the lipid bilayer (Kabir and Ahmad, 2013). When two ATP molecules bind with two NBDs, conformational changes in TMDs induce the extracellular opening and intracellular closing of these cavities, allowing the bound drugs to be effluxed from the cell. The hydrolysis of NBDs bound ATP resets the efflux pump in drug-binding mode, completing one cycle that is repeated to efflux the drug from the fungal cell, thus making it drug-resistant. While this mechanism has been presumed from the ABC transporter Sav1866 of *Staphylococcus aureus* whose crystal structure is available together with AMP-PNP in the absence of an ABC transporter crystal structure (Dawson and Locher, 2007; Pinkett *et al*., 2007), over expression of Cdr1p, Cdr2p, and Mdr1p is the main reason for azole resistance in clinical isolates of *Candida* spp. Cdr1p and Cdr2p also implicate drug resistance to topical antifungal agents, such as amorrolfine and terbinafine.

6.2 Target site mutations

In antifungal drug-resistant clinical strains of *Candida* spp., mutations are observed in several genes. However, a particular mutation in a specific gene is necessary for a strain to become resistant to a particular drug. For instance, a mutation in the ERG11 gene can reduce the binding of azoles to lanosterol 14-α-demethylase, resulting in increased resistance to azoles. *Candida* strains with a mutation in the ERG11 gene also exhibit cross-resistance to different azoles (Lamb *et al*., 2000). Mutations in the FUR1 gene, which encodes uracil phosphoribosyl transferase, prevent the conversion of 5FU to FdUMP, leading to 5FC resistance in *Candida* strains. This mutation affects the uptake of 5FC or its conversion to 5FU and its incorporation into newly synthesizing nucleic acid (Florent *et al*., 2009). The FUR1 gene mutation occurs at the 301 bp position, resulting in an amino acid change from arginine to cysteine at the 101 bp position in Fur1p.

Mutations in the FCA1 gene, which encodes the cytosine deaminase enzyme, are also implicated in 5FC resistance in *C. albicans*, resulting in amino acid changes from glycine and serine to aspartate and leucine, respectively, at positions 28 and 29 of this enzyme. The resistance to 5FC due to the FCA1 gene mutation was also observed in other *Candida* spp., such as *C. glabrata*, *C. dubliniensis*, and *C. lusitaniae*. Similarly, mutations in the FKS1 gene, which encodes a subunit of the β-1, 3-glucan synthase complex, can cause resistance to echinocandin drugs (De Oliveira Santos *et al*., 2018). *Candida* spp. are highly resistant to antifungal drugs and possess virulent features, such as the ability to form biofilms with other species, making them a serious risk to human health (Kiran *et al*., 2021). Additionally, as fungi are eukaryotic organisms that parasitize eukaryotic hosts, developing safe and broad-spectrum antifungal drugs is challenging due to the limited physiological differences between the two (Liu *et al*., 2017). Therefore, there is a pressing need for new antifungals with a novel mode of action that can avoid cross-resistance and cross-toxicities. Combination therapies can be a better option as they are capable of preventing the emergence of drug resistance and increasing the efficacy of antifungals.

7. Lactic acid bacteria and its role in management of *C. albicans* infections

Before the 20th century, the terms “milk souring bacteria” and “lactic acid producing bacteria” were used interchangeably, leading to

confusion in the scientific community. However, in the early 1900s, the term Lactic Acid Bacteria (LAB) gained acceptance (van Reenen and Dicks, 2011) and has since been used to refer to a group of closely related bacteria that share physiological, morphological, and metabolic similarities. LAB are classified based on their morphology, growth at certain temperatures, sugar utilization range, and glucose fermentation modes. Phylogenetic analysis has been critical in characterizing LAB, with rRNA sequencing providing the most accurate and powerful technique for determining their relationship with other microorganisms. Specifically, the analysis of long rRNA sequences (~1500 bases of 16S rRNA) can be used to determine the phylogenetic positions of species and genera. This technique has led to the emergence of new genera descriptions and contributed to our understanding of LAB phylogeny and classification. (Tamang *et al*., 2008; Hovarth *et al*., 2009; Pang *et al*., 2011). LAB are Gram-positive, non-motile, non-spore-forming, and typically cocci or rod-shaped. They are part of the human microbiome and are known for their ability to produce lactic acid as their major end product through carbohydrate fermentation (Kiran *et al*., 2021). Despite their lack of cytochromes and porphyrins synthesis, LAB generates ATP through sugar fermentation and does not require oxygen for energy production. Moreover, LAB are able to grow under anaerobic conditions but are also aerotolerant anaerobes due to the presence of peroxidases (Stieglmeier *et al*., 2009), which protect them from oxygen byproducts.

There are two types of sugar fermentation pathways present in LAB: glycolysis, which results in the exclusive production of lactic acid under standard conditions, also known as homolactic fermentation; and the 6-phosphogluconate pathway, which produces other metabolites in addition to lactic acid, such as acetate, ethanol, and CO_2. This pathway is referred to as heterolactic fermentation (Abbott *et al*., 2009). The diverse metabolic capacity of LAB allows them to adapt to different environmental conditions. In addition to their ability to ferment sugars, LAB are known to inhibit the growth of *Candida* spp. through various mechanisms, including the production of antagonistic metabolites and competition for adhesion sites (Suissa *et al*., 2021). In the human body, LAB produce organic acids, carbon peroxide, hydrogen peroxide, diacetyl, antimicrobial substances (such as bacteriocins), and adhesion inhibitors (such as biosurfactants) that exhibit antifungal activity (Khalid, 2011; Kiran *et al*., 2021). These metabolites naturally suppress filamentation, a key virulence feature of *C. albicans*.

Moreover, the use of *Lactobacilli*-derived probiotic bacteria has emerged as a new technique for the therapy of *Candida* infections in recent years. probiotic *Lactobacillus* strains such as *L. crispatus*, *L. acidophilus* and *L. paracasei* show fungicidal or candidacidal effects. The candidacidal effects of *L. crispatus*, which is thought to contribute to vaginal microbiota regulation by competing with other microflora for colonisation of vaginal epithelial cells and releasing H_2O_2 and other metabolites that promote intracellular antifungal activity (Li *et al*., 2019). Viable *L. acidophilus* are able to suppress the viable *C. albicans* in the alimentary canal, stomach and intestine (Wagner *et al*., 2000). Antifungal properties of orally derived probiotic *L. paracasei* are decreased expression of virulence genes (ALS3, HWP1, EFG1 and CPH1), biofilm deterrence and retardation of hyphal formation resulting in inhibition of *C. albicans* growth (Ribeiro *et al*., 2020).

7.1 Metabolites produced by LAB

Probiotic bacteria, including *Lactobacilli*, are well-known for their positive impact on human interference factors due to their production of various antimicrobial agents, such as organic acids, hydrogen peroxide, carbon peroxide, diacetyl, low molecular weight antimicrobial substances, bacteriocins, and adhesion inhibitors like biosurfactants (Merk *et al*., 2005; Gupta and Garg, 2009). LAB produces various metabolic byproducts that support their persistence in the host and provide them with a survival advantage over other pathogens (Zangl *et al*., 2020). For instance, *Lactobacillus* spp. generate metabolites such as organic acids (acetic and lactic acid), reactive oxygen species (H_2O_2), bacteriocins, biosurfactants, and other compounds.

7.1.1 Organic acids

Lactobacilli are known to produce short-chain aliphatic organic acids, including acetic and lactic acid. In the vaginal environment, the concentration of acetic acid is typically low, ranging from 1-4 mM, as this environment is anaerobic or micro-aerobic, and acetic acid is mainly produced under aerobic conditions. However, the concentration of acetic acid may increase during bacterial vaginosis. In contrast, the concentration of lactic acid is typically high, ranging from 110 mM (O'Hanolan *et al*., 2013). Lactic acid is produced through anaerobic respiration and helps to lower the pH in the vaginal tract. It has been shown to inhibit the growth of *C. albicans* at a low pH. The production rate and capability of lactic acid are strain-specific and only an elevated concentration of lactic acid can efficiently inhibit fungal growth (Matsubara *et al*., 2016). This is proposed because inhibition of *C. glabrata* or *C. albicans* is not observed with the lactic acid concentration produced by the supernatant of an *L. rhamnosus* strain (Lourenco *et al*., 2019). However, supernatants of *L. rhamnosus*, *L. acidophilus*, and *L. casei* show antifungal activity against *Candida spp.* after prolonged incubation, during which lactic acid accumulates in the medium (Matsubara *et al*., 2016).

Studies show that *Lactobacillus* remains the dominant bacterial species during vulvovaginal candidiasis (VVC), but the composition of their strains could vary, leading to a reduction in lactic acid concentration and other metabolites. This, in turn, permits *Candida* growth in the vaginal tract (Zangl *et al*., 2020). Organic acids, such as lactic and acetic acid, have been shown to increase the efficacy of azoles against *C. albicans* at physiological concentrations. At high concentrations, they also increase the efficacy of azoles against *C. glabrata* (Lourenco *et al*., 2019). It is believed that the uptake of azoles into yeast cells is increased due to the plasma membrane perturbation caused by these undissociated organic acids (Mira *et al*., 2010). While the overall concentration of organic acids is not enough to have a fungistatic effect on its own, they could still be used to improve the treatment of *Candida* infections by enhancing the efficacy of azole antifungal drugs.

7.1.2 Hydrogen peroxide

H_2O_2 production is a very important feature of *Lactobacillus* spp. against bacterial infections. H_2O_2 plays minor role in the defense of *Candida* against *Lactobacillus* spp. in the micro aerobic environment of vagina because the vaginal conditions are hypoxic and *Lactobacillus* spp. produce H_2O_2 predominantly under aerobic conditions (Tachedjian *et al*., 2017). Physiological concentration reached in

Lactobacillus cultures (<100 µM) cause no harm to *Candida* spp., BV-associated bacteria and *lactobacilli* but H_2O_2 concentration like 10 mM are shown to be harmful to *Lactobacillus* spp. in vagina, thus could harm the *Candida* spp. too. *C. albicans* growth is also inhibited by non-H_2O_2 producers like *L. rhamnosus* GR-1 and H_2O_2 producer *L. reuteri* RC-14 both (Kohler *et al*., 2012). Similar results were seen with *C. glabrata* (Chew *et al*., 2015) because most of *C. glabrata* isolates are very tolerant against reactive oxygen species like H_2O_2.

H_2O_2 production is an important defense mechanism employed by *Lactobacillus* spp. against bacterial infections. However, in the hypoxic vaginal environment where *Lactobacillus* spp. primarily reside, H_2O_2 production is not a significant factor in the defense against *Candida* spp. This is because *Lactobacillus* spp. predominantly produce H_2O_2 under aerobic conditions (Tachedjian *et al*., 2017). In addition, H_2O_2 concentrations that are typically reached in *Lactobacillus* cultures (<100 µM) pose no threat to *Candida* spp., BV-associated bacteria, or lactobacilli. However, higher concentrations of H_2O_2 (*e.g.*, 10 mM) have been shown to be harmful to *Lactobacillus* spp. in the vaginal environment, which could potentially harm *Candida* spp. as well. Interestingly, some non-H_2O_2 producers, such as *L. rhamnosus* GR-1, are also able to inhibit the growth of *C. albicans*, as are H_2O_2 producers like *L. reuteri* RC-14 (Kohler *et al*., 2012). Similarly, *C. glabrata* has been found to be tolerant to reactive oxygen species, including H_2O_2, which may explain why the growth of this species is not inhibited by *Lactobacillus* spp. (Zangl *et al*., 2020).

7.1.3 Bacteriocins

LAB produce a range of antifungal factors, including small biomolecules such as bacteriocins and biosurfactants. Bacteriocins are proteinaceous substances that can inhibit the growth of the same or closely related species (Peleg *et al*., 2010), while bacteriocin-like substances (which are very similar to bacteriocins) have a broader inhibitory range and can target species such as Gram-positive bacteria, Gram-negative bacteria, or fungi. For instance, *Lactobacillus pentosus* produces the bacteriocin-like peptide pentocin TV35b, which has a fungistatic effect on *C. albicans* (Rodrigues *et al*., 2006). The pentocin TV35b is the bacteriocin-like peptide that has been reported to have this effect.

7.1.4 Biosurfactants

In recent years, biosurfactants have become increasingly important in biotechnology for industrial and medical applications (Rivardo *et al*., 2009). When biosurfactants are adsorbed onto a substrate surface, they modify the surface's hydrophobicity and interfere with microbial adhesion and desorption processes. Probiotic bacteria release biosurfactants *in vivo* as a defense mechanism against fungal strains colonizing in the gastrointestinal and urogenital tracts (van Hoogmoed *et al*., 2004). The initial step of fungal infection is adhesion to mucosa and biosurfactants produced by *Lactobacillus* spp. have been shown to reduce adhesion between the epithelial cell wall and the pathogen, thus reducing the risk of fungal infection. Biosurfactants produced by *L. jensenni* and *L. gasseri* have been found to inhibit the biofilm formation of *C. krusei, C. tropicalis* and *C. albicans* on polystyrene plates (Morais *et al*., 2017). Biosurfactant CV8LAC, produced by *L. brevis*, has the potential to decrease the adhesion of *C. albicans* and biofilm formation on pre-coated medical grade silicone **(**Ceresa *et al*., 2015), making it a potential coating material for medical devices to minimize *Candida* infections.

C. albicans induces cellular endocytosis in vaginal epithelial cells by adhering and initiating morphological changes in cells. However, *L. crispatus* has been found to reduce adhesion, proliferation, and hyphal formation of *Candida* in infected cells (Niu *et al*., 2017). Pre-incubation with *L. crispatus* L1 produced extracellular polysaccharides that reduced *C. albicans* adhesion to Vk2/E6E7, which is similar to cell-dependent adhesion reduction by pre-incubated *L. crispatus* L1. Extracellular polysaccharides may be a potential new coating agent. Adhesion reduction depending on cell is due to *Candida* and *Lactobacillus* spp. coaggregation, which is a characteristic of early biofilm formation involving adhesion-receptor interaction between the cell surfaces of microbes. Therefore, proper adhesion of *Candida* to mucosal surfaces is influenced not only by competition for binding sites but also by changes in epithelial cell surfaces and the adhesion ability of the pathogen itself (Parolin *et al*., 2015).

7.2 Inhibition of *C. albicans* by LAB

Lactobacillus spp. have been shown to influence the morphology of *C. albicans*. Tissue adhesion, biofilm formation and hyphal morphogenesis are the required events in *C. albicans* pathogenesis. Thus, many of the identified probiotic interference mechanisms of LAB are associated with inhibition of one or more of these processes. It is known that polysaccharides present on bacterial surface (lipopolysaccharides, capsular polysaccharides and exopoly saccharides) are involved in probiotic activity of certain *Lactobacillus* strains. *L. rhamnosus* antagonize *C. albicans* by harboring galactose rich exopolysaccharides on its surface which contribute its stable colonization in host organs. Galactose rich exopolysaccharides blocks the *C. albicans* adhesion to several host epithelial cells by binding with lectin like adhesins present in *C. albicans*. The anti-adhesive effect of *L. rhamnosus* is due to co-aggregation with *C. albicans* rather than competitive exclusion. Furthermore, *L. rhamnosus* exopolysaccharides decrease the ratio of hyphae to yeast cells (Zeise *et al*., 2021).

Co-culture with *L. paracasei* inhibits the formation of hyphae in *Candida* (de Burros *et al*., 2018). Moreover, the interaction of *C. albicans* with *Lactobacillus* alters the pattern of gene expression associated with yeast-to-hyphae transition, adhesion, and biofilm formation. For instance, treatment with *L. paracasei* supernatant suppresses genes such as ALS3, HWP1, and EFG1 in *C. albicans* cells (James *et al*., 2016). EFG1, which regulates the expression of genes like HWP1, SAP, and ALS3 involved in the transition of yeast to hyphal form, is inhibited by *Lactobacillus*. Notably, the yeast form of *Candida* exhibits lower levels of adhesion and biofilm formation as compared to its hyphal form (Hall and Noverr, 2017). After interacting with *L. paracasei*, the expression of a yeast-form-associated gene, YWP1, is induced. In addition, the pH-responsive gene PHR1, which codes for a glucan remodeling enzyme supporting hyphal growth, is down-regulated when *C. albicans* is co-cultured with *L. reuteri* RC-14 and *L. rhamnosus* GR-1 (Calderon *et al*., 2010), suggesting that *Lactobacillus* spp. help to maintain *C. albicans* in a less invasive form and inhibit fungal overgrowth. Taken together, these findings highlight the potential of *Lactobacillus* spp. as a novel probiotic, with the specific strain having a crucial role in managing *C. albicans*.

8. Conclusion

Candidiasis is a serious and widespread disease due to limited antifungal options and increasing drug resistance. Combination therapy is a promising approach to enhance treatment outcomes while reducing toxicity. Innovative technologies targeting *Candida* spp. biofilm growth has significant potential for improving clinical practice and preventive medicine. *Lactobacilli* have shown promise as a novel strategy for controlling fungal infections, inhibiting *Candida* growth through multiple mechanisms. Specific LAB strains, including *L. crispatus*, *L. acidophilus*, and *L. paracasei*, have been identified as potential probiotic candidates for preventing and treating *Candida* infections. Administration of LAB-based probiotics can improve the efficacy of conventional antifungal therapy and reduce the risk of recurrent infections. Overall, LAB-based probiotics offer a natural and safe alternative for the management of *Candida* infection, but further research is needed to identify the most effective strains and optimize their application methods for clinical trials.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Abbott, D.A.; Van Den Brink, J.; Minneboo, I.M.; Pronk, J.T. and Van Maris, A.J. (2009). Anaerobic homolactic fermentation with *Saccharomyces cerevisiae* results in depletion of ATP and impaired metabolic activity. FEMS Yeast research, **9**(3):349-357. doi: 10.1111/j.1567-1364.2009. 00506.x.

Albrecht, A.; Felk, A.; Pichova, I.; Naglik, J.R.; Schaller, M.; de Groot, P.; Maccallum, D.; Odds, F.C.; Schafer, W.; Klis, F.; Monod, M. and Hube, B. (2006). Glycosylphosphatidylinositol-anchored proteases of *Candida albicans* target proteins necessary for both cellular processes and host-pathogen interactions. The Journal of Biological Chemistry, **281**(2):688-694. doi: 10.1074/jbc.M509297200.

Al-Dhaheri, R.S. and Douglas, L.J. (2010). Apoptosis in *Candida* biofilms exposed to amphotericin B. Journal of Medical Microbiology, **59**(2):149-157. doi: 10.1099/jmm.0.015784-0.

Alghaith, A.F.; Alshehri, S.; Alhakamy, N.A. and Hosny, K.M. (2021). Development, optimization and characterization of nanoemulsion loaded with clove oil-naftifine antifungal for the management of tinea. Drug Delivery, **28**(1):343-356. doi:10.1080/10717544.2021. 1879314

Almeida, R.S.; Wilson, D. and Hube, B. (2009). *Candida albicans* iron acquisition within the host. FEMS Yeast Research, **(7)**:1000-1012. doi:10.1111/j.1567-1364.2009. 00570.x.

Barchiesi, F.; Orsetti, E.; Gesuita, R.; Skrami, E.; Manso, E. and Candidemia Study Group (2016). Epidemiology, clinical characteristics, and outcome of candidemia in a tertiary referral center in Italy from 2010 to 2014. Infection, **44**:205-213. doi: 10.1007/s15010-015-0845-z.

Berman, J. and Sudbery, P.E. (2002). *Candida Albicans*: a molecular revolution built on lessons from budding yeast. Nature reviews. Genetics, **3**(12):918-930. doi: 10.1038/nrg948.

Bhagat, J.; Singh, N.; Nishimura, N. and Shimada, Y. (2020). A comprehensive review on environmental toxicity of azole compounds to fish. Chemosphere, 128335. doi:10.1016/j.chemosphere. 2020.128335

Brand, A.; Vacharaksa, A.; Bendel, C.; Norton, J.; Haynes, P.; Henry-Stanley, M.; Wells, C.; Ross, K.; Gow, N.A. and Gale, C.A. (2008). An internal polarity landmark is important for externally induced hyphal behaviors in *Candida albicans*. Eukaryotic Cell, **7**(4):712-720. doi: 10.1128/ EC.00453-07.

Brock, M. (2009). Fungal metabolism in host niches. Current Opinion in Microbiology, **12**(4):371-376. doi: 10.1016/j.mib.2009.05.004.

Calderon, J.; Zavrel, M.; Ragni, E.; Fonzi, W.A.; Rupp, S. and Popolo, L. (2010). PHR1, a pH-regulated gene of *Candida albicans* encoding a glucan-remodeling enzyme, is required for adhesion and invasion. Microbiology, **156**(8):2484-2494. doi: 10.1099/mic.0.038000-0.

Cannon, R.D.; Lamping, E.; Holmes, A.R.; Niimi, K.; Baret, P.V.; Keniya, M.V.; Tanabe, K.; Niimi, M.; Goffeau, A. and Monk, B.C. (2009). Efflux-mediated antifungal drug resistance. Clinical Microbiology Reviews, **22**(2):291-321 doi:10.1128/CMR.00051-08.

Cassone, A.; De Bernardis, F. and Santoni, G. (2007). Anticandidal immunity and vaginitis: novel opportunities for immune intervention. Infection and Immunity, **75**(10):4675-4686. doi: 10.1128/IAI. 00083-07.

Ceresa, C.; Tessarolo, F.; Caola, I.; Nollo, G.; Cavallo, M.; Rinaldi, M. and Fracchia, L. (2015). Inhibition of *Candida albicans* adhesion on medical grade silicone by a *Lactobacillus* derived biosurfactant. Journal of Applied Microbiology, **118**(5):1116-1125. doi: 10.1111/jam.12760.

Cerqueira, F.; Maia, M.; Gabriel, C.; Medeiros, R.; Cravo, S.; Ribeiro, A.I. and Pinto, E. (2021). Mechanism of antifungal activity by 5-aminoimidazole-4-carbohydrazonamide derivatives: Against *Candida albicans* and *Candida krusei*. Antibiotics, 10(2), 183. doi:10.3390/antibiotics10020183

Chew, S.Y.; Cheah, Y.K.; Seow, H.F.; Sandai, D. and Than, L.T.L. (2015). Probiotic *Lactobacillus rhamnosus* GR 1 and *Lactobacillus reuteri* RC 14 exhibit strong antifungal effects against vulvovaginal candidiasis causing *Candidaglabrata* isolates. Journal of Applied Microbiology, **118**(5):1180-1190. doi: 10.1111/jam.12772.

Cleary, I.A.; Reinhard, S.M.; Miller, C.L.; Murdoch, C.; Thornhill, M.H.; Lazzell, A.L.; Monteagudo, C.; Thomas, D.P. and Saville, S.P. (2011). *Candida albicans* adhesin Als3p is dispensable for virulence in the mouse model of disseminated candidiasis. Microbiology (Reading, England), **157**(Pt 6):1806-1815. doi:10.1099/mic.0.046326-0.

Costerton, J. (1995). Overview of microbial biofilms. Journal of Industrial Microbiology, **15**(3):137-140. doi: 10.1007/bf01569816.

Dalle, F.; Wachtler, B.; L'Ollivier, C.; Holland, G.; Bannert, N.; Wilson, D.; Labruere, C.; Bonnin, A. and Hube, B. (2010). Cellular interactions of *Candida albicans* with human oral epithelial cells and enterocytes. Cellular Microbiology, **12**(2):248-271. doi:10.1111/j.1462-5822.2009.013 94.x.

Davis, D.; Edwards, J.E.; Mitchell, A.P. and Ibrahim, A.S. (2000). *Candida albicans* RIM101 pH response pathway is required for host-pathogen interactions. Infection and Immunity, **68**(10):5953-5959. doi:10. 1128/iai.68.10.5953-5959.2000.

Davis, D.A. (2009). How human pathogenic fungi sense and adapt to pH: the link to virulence. Current Opinion in Microbiology, **12**(4):365-370. doi:10.1016/j.mib.2009.05.006.

Dawson, R.J. and Locher, K.P. (2007). Structure of the multidrug ABC transporter Sav1866 from *Staphylococcus aureus* in complex with AMP-PNP. FEBS Letters, **581**(5):935-938. doi: 10.1016/j.febslet. 2007.01.073.

de Barros, P.P.; Scorzoni, L.; de Camargo Ribeiro, F.; de Oliveira Fugisaki, L.R.; Fuchs, B.B.; Mylonakis, E.; Jorge, A.O.C.; Junqueira, J.C. and Rossoni, R.D. (2018). *Lactobacillus paracasei* 28.4 reduces *in vitro* hyphae formation of *Candida albicans* and prevents the filamentation in an experimental model of *Caenorhabditis elegans*. Microbial Pathogenesis, **117**:80-87. doi: 10.1016/j.micpath.2018.02.01.

De Oliveira Santos, G.C.; Vasconcelos, C.C.; Lopes, A.J.; de Sousa Cartagenes, M. D. S.; Filho, A.K.; do Nascimento, F.R.; Ramos, R.M.; Pires, E.R.; de Andrade, M.S.; Rocha, F.M. and de Andrade Monteiro, C. (2018). *Candida* infections and therapeutic strategies: Mechanisms of action for traditional and alternative agents. Frontiers in Microbiology, **9**. doi:10.3389/fmicb.2018.01351.

Dongari-Bagtzoglou, A.; Villar, C.C. and Kashleva, H. (2005). *Candida albicans*-infected oral epithelial cells augment the antifungal activity of human neutrophils *in vitro*. Medical Mycology, **43**(6):545-549. doi: 10.1080/13693780500064557.

Eyles, S.J. and Gierasch, L.M. (2010). Nature's molecular sponges: small heat shock proteins grow into their chaperone roles. Proceedings of the National Academy of Sciences, **107**(7):2727-2728. doi:10.1073/pnas.0915160107.

Fanning, S. and Mitchell, A.P. (2012). Fungal biofilms. PLoS Pathogens, **8**(4):e1002585. doi: 10.1371/journal.ppat.1002585.

Finkel, J.S. and Mitchell, A.P. (2011). Genetic control of *Candida albicans* biofilm development. Nature reviews. Microbiology, **9**(2):109-118. doi: 10.1038/nrmicro2475.

Florent, M.; Noel, T.; Ruprich-Robert, G.; Da Silva, B.; Fitton-Ouhabi, V.; Chastin, C.; Papon, N. and Chapeland-Leclerc, F. (2009). Nonsense and missense mutations in FCY2 and FCY1 genes are responsible for flucytosine resistance and flucytosine-fluconazole cross-resistance in clinical isolates of *Candida lusitaniae*. Antimicrobial Agents and Chemotherapy, **53**(7):2982-2990. doi: 10.1128/AAC.00880-08.

Frohner, I.E.; Bourgeois, C.; Yatsyk, K.; Majer, O. and Kuchler, K. (2009). *Candida albicans* cell surface superoxide dismutases degrade host derived reactive oxygen species to escape innate immune surveillance. Molecular Microbiology, **71**(1):240-252 doi:10.1111/j.1365-2958.2008.06528.x.

Fu, M.S.; De Sordi, L. and Muhlschlegel, F.A. (2012). Functional characterization of the small heat shock protein Hsp12p from *Candida albicans*. PloS one, **7**(8): e42894. doi: 10.1371/journal.pone. 0042894.

Gacser, A.; Stehr, F.; Kroger, C.; Kredics, L.; Schafer, W. and Nosanchuk, J.D. (2007). Lipase 8 affects the pathogenesis of *Candida albicans*. Infection and Immunity, **75**(10):4710-4718. doi: 10.1128/IAI.00372-07.

Garcia, M.C.; Lee, J.T.; Ramsook, C.B.; Alsteens, D.; Dufrene, Y.F. and Lipke, P.N. (2011). A role for amyloid in cell aggregation and biofilm formation. PloS one, **6**(3):e17632. doi: 10.1371/journal.pone. 0017632.

Giri, S. and Kindo, A.J. (2012). A review of *Candida* species causing blood stream infection. Indian Journal of Medical Microbiology, **30**(3):270-278. doi: 10.4103/0255-0857.99484.

Ghosh, S.; Navarathna, D.H.; Roberts, D.D.; Cooper, J.T.; Atkin, A.L.; Petro, T.M. and Nickerson, K.W. (2009). Arginine-induced germ tube formation in *Candida albicans* is essential for escape from murine macrophage line RAW 264.7. Infection and Immunity, **77**(4):1596-1605. doi:10.1128/IAI.01452-08.

Guery, B.P.; Arendrup, M.C.; Auzinger, G.; Azoulay, E.; Borges Sa, M.; Johnson, E.M.; Muller, E.; Putensen, C.; Rotstein, C.; Sganga G.; Venditti, M.; Crespo, R.Z. and Kullberg, B.J. (2009). Management of invasive candidiasis and candidemia in adult non-neutropenic intensive care unit patients: Part II. Treatment. Intensive Care Medicine, **35**:206-214. doi.org/10.1007/s00134-008-1339-6.

Gupta, V. and Garg, R. (2009). Probiotics. Indian Journal of Medical Microbiology, **27**(3):202-209. doi: 10.4103/0255-0857.53201.

Hall, R.A. and Noverr, M.C. (2017). Fungal interactions with the human host: Exploring the spectrum of symbiosis. Current Opinion in Microbiology, **40**:58-64. doi: 10.1016/j.mib.2017.10.020.

Heymann, P.; Gerads, M.; Schaller, M.; Dromer, F.; Winkelmann, G. and Ernst, J.F. (2002). The siderophore iron transporter of *Candida albicans* (Sit1p/Arn1p) mediates uptake of ferrichrome-type siderophores and is required for epithelial invasion. Infection and Immunity, **70**(9):5246-5255. doi:10.1128/IAI.70.9.5246-5255.2002.

Hood, M.I. and Skaar, E.P. (2012). Nutritional immunity: transition metals at the pathogen-host interface. Nature reviews. Microbiology, **10**(8):525-537. doi:10.1038/nrmicro2836.

Hornby, J.M.; Jensen, E.C.; Lisec, A.D.; Tasto, J.J.; Jahnke, B.; Shoemaker, R.; Dussault, P.; and Nickerson, K.W. (2001). Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. Applied and Environmental Microbiology, **67**(7):2982-2992. doi: 10.1128/AEM.67.7.2982-2992.2001.

Horvath, P.; Coute-Monvoisin, A.C.; Romero, D.A.; Boyaval, P.; Fremaux, C. and Barrangou, R. (2009). Comparative analysis of CRISPR loci in lactic acid bacteria genomes. International Journal of Food Microbiology, **131**(1):62-70. doi: 10.1016/j.ijfoodmicro.2008.05.030.

Houst, J.; Spizek, J. and Havlicek, V. (2020). Antifungal Drugs. Metabolites, 10:106.

Iorio, E.; Torosantucci, A.; Bromuro, C.; Chiani, P.; Ferretti, A.; Giannini, M.; Cassone, A. and Podo, F. (2008). *Candida albicans* cell wall comprises a branched β-d-(1<6)-glucan with β-d-(1<3)-side chains. Carbohydrate Research, **343**(6):1050-1061. doi: 10.1016/j.carres.2008.02.020.

James, K.M.; MacDonald, K.W.; Chanyi, R.M.; Cadieux, P.A. and Burton, J.P. (2016). Inhibition of *Candida albicans* biofilm formation and modulation of gene expression by probiotic cells and supernatant. Journal of Medical Microbiology, **65**(4):328-336. doi: 10.1099/jmm.0.000226.

Kabir, M.A. and Ahmad, Z. (2013). *Candida* Infections and Their Prevention. ISRN Preventive Medicine, pp:1-13. doi:10.5402/2013/763628.

Kanwar, K.; Pandey, R.; Gezici, S. and Azmi, W. (2019). Enzymes as competent tool for efficient management of pathogen's biofilms. Ann. Phytomed., **8**(1):70-81. doi: 10.21276/ap.2019.8.1.8.

Khalid, K. (2011). An overview of lactic acid bacteria. International Journal of Biosciences, **1**(3):1-13.

Kiran; Kaur, A. and Azmi, W. (2021). Multidrug-resistant pathogens and their plasmid curing by lactic acid bacteria. Ann. Phytomed., **10**(2):101-112 doi: 10.21276/ap.2021.10.2.14.

Kohler, G.A.; Assefa, S. and Reid, G. (2012). Probiotic interference of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 with the opportunistic fungal pathogen *Candida albicans*. Infectious Diseases in Obstetrics and Gynecology, pp:1-14 doi: 10.1155/2012/636474.

Kumamoto C.A. (2008). Molecular mechanisms of mechanosensing and their roles in fungal contact sensing. Nature reviews. Microbiology, **6**(9):667-673. doi:10.1038/nrmicro1960.

Kumar, N.; Gupta, P.; Gautam, P. and Rai, N. (2012). An emergence hope to combat *Candida albicans*: Plant based therapeutics. Biotechnology International **5**(3):85-114.

Lamb, D.C.; Kelly, D.E.; White, T.C. and Kelly, S.L. (2000). The R467K amino acid substitution in *Candida albicans* sterol 14α-demethylase causes drug resistance through reduced affinity. Antimicrobial Agents and Chemotherapy, **44**(1):63-67. doi: 10.1128/AAC.44.1.63-67.2000.

Leach, M.D.; Stead, D.A.; Argo, E. and Brown, A.J. (2011). Identification of sumoylation targets, combined with inactivation of SMT3, reveals the impact of sumoylation upon growth, morphology, and stress resistance in the pathogen *Candida albicans*. Molecular Biology of the Cell, **22**(5):687-702. doi:10.1091/mbc. e10-07-0632.

Lilic, D. (2002). New perspectives on the immunology of chronic mucocutaneous candidiasis. Current Opinion in Infectious Diseases, **15**(2):143-147.

Li, T.; Liu, Z.; Zhang, X.; Chen, X. and Wang, S. (2019). Local probiotic *Lactobacillus crispatus* and *Lactobacillus delbrueckii* exhibit strong antifungal effects against *Vulvovaginal candidiasis* in a rat model. Frontiers in Microbiology, 10. doi:10.3389/fmicb.2019.01033

Liu, F.; Liao, Q. and Liu, Z. (2006). Mannose-binding lectin and vulvovaginal candidiasis. International Journal of Gynecology and Obstetrics, **92**(1):43-47. doi.org/10.1016/j.ijgo.2005.08.024.

Liu, X.; Ma, Z.; Zhang, J. and Yang, L. (2017). Antifungal compounds against *Candida* infections from traditional Chinese medicine. BioMed Research International, 1-12 doi: 10.1155/2017/4614183.

Lorenz, M.C.; Bender, J.A. and Fink, G.R. (2004). Transcriptional response of *Candida albicans* upon internalization by macrophages. Eukaryotic Cell, **3**(5):1076-1087. doi:10.1128/EC.3.5.1076-1087.2004.

Lourenco, A.; Pedro, N.A.; Salazar, S.B. and Mira, N.P. (2019). Effect of acetic acid and lactic acid at low pH in growth and azole resistance of *Candida albicans* and *Candida glabrata*. Frontiers in Microbiology, **9**:3265. doi: 10.3389/fmicb.2018.03265.

Matsubara, V.H.; Wang, Y.; Bandara, H.M.; Mayer, M.P. and Samaranayake, L.P. (2016). Probiotic *lactobacilli* inhibit early stages of *Candida albicans* biofilm development by reducing their growth, cell adhesion, and filamentation. Applied Microbiology Biotechnology, **100**(14):6415-6426. doi: 10.1007/s00253-016-7527-3.

Matsumori, N.; Tahara, K.; Yamamoto, H.; Morooka, A.; Doi, M.; Oishi, T. and Murata, M. (2009). Direct interaction between amphotericin B and ergosterol in lipid bilayers as revealed by 2H NMR spectroscopy. Journal of the American Chemical Society, **131**(33):11855-11860. doi: 10.1021/ja9033473.

Mavor, A.L.; Thewes, S. and Hube, B. (2005). Systemic fungal infections caused by *Candida* species: epidemiology, infection process and virulence attributes. Current Drug Targets, **6**(8):863-874. doi:10.2174/138945005774912735.

Mayer, F.L.; Wilson, D.; Jacobsen, I.D.; Miramon, P.; Grobe, K. and Hube, B. (2012). The Novel *Candida albicans* Transporter Dur31 Is a Multi-Stage Pathogenicity Factor. PLoS Pathogens, **8**(3):e1002592. doi: 10.1371/journal.ppat.1002592.

Mayer, F.L.; Wilson, D. and Hube, B. (2013). *Candida albicans* pathogenicity mechanisms. Virulence, **4**(2):119-128. doi:10.4161/viru.22913.

Merk, K.; Borelli, C. and Korting, H.C. (2005). *Lactobacilli*–bacteria–host interactions with special regard to the urogenital tract. International Journal of Medical Microbiology, **295**(1):9-18. doi: 10.1016/j.ijmm.2004.11.006.

Miao, J.; Willems, H.M.E. and Peters, B.M. (2020). Exogenous reproductive hormones nor *Candida albicans* colonization alter the near neutral mouse vaginal pH. Infection and immunity, doi:10.1128/iai.00550-20

Mira, N.P.; Teixeira, M.C. and Sa-Correia, I. (2010). Adaptive response and tolerance to weak acids in *Saccharomyces cerevisiae*: a genome-wide view. OMICS: A Journal of Integrative Biology, **14**(5):525-540. doi: 10.1089/omi.2010.0072.

Morais, I.M.C.; Cordeiro, A.L.; Teixeira, G.S.; Domingues, V.S.; Nardi, R.M.D.; Monteiro, A.S.; Alves, R.J.; Squeira, E.P. and Santos, V.L. (2017). Biological and physicochemical properties of biosurfactants produced by *Lactobacillus jensenii* P 6A and *Lactobacillus gasseri* P 65. Microbial Cell Factories, **16**:1-15. doi: 10.1186/s12934-017-0769-7.

Morschhauser, J. (2010). Regulation of multidrug resistance in pathogenic fungi. Fungal Genetics and Biology, **47**(2):94-106. doi: 10.1016/j.fgb.2009.08.002.

Mortale, S.P. and Karuppayil, S.M. (2018). Review on Combinatorial Approach for Inhibiting *Candida albicans* Biofilm. American Journal of Clinical Microbiology and Antimicrobials, **1**(5):1022.

Mouri, R.; Konoki, K.; Matsumori, N.; Oishi, T. and Murata, M. (2008). Complex formation of amphotericin B in sterol-containing membranes as evidenced by surface plasmon resonance. Biochemistry, **47**(30):7807-7815. doi:10.1021/bi800334p.

Murciano, C.; Moyes, D.L.; Runglall, M.; Tobouti, P.; Islam, A.; Hoyer, L.L. and Naglik, J.R. (2012). Evaluation of the role of *Candida albicans* agglutinin-like sequence (Als) proteins in human oral epithelial cell interactions. PloS one, **7**(3):e33362. doi: 10.1371/journal.pone.0033362.

Narberhaus, F. (2002). β-Crystallin-type heat shock proteins: socializing minichaperones in the context of a multichaperone network. Microbiology and Molecular Biology Reviews, **66**(1):64-93. doi:10.1128/MMBR.66.1.64-93.2002.

Netea, M.G; Vonk, A.G; van den Hoven, M.; Verschueren, I.; Joosten, L.A.; van Krieken, J.H.; van Den Berg, W.B.; Van der Meer, J.W. and Kullberg, B.J. (2003). Differential role of IL 18 and IL 12 in the host defense against disseminated *Candida albicans* infection. European Journal of Immunology, **33**(12):3409-3417. doi: 10.1002/eji.200323737.

Netea, M.G; Gijzen, K.; Coolen, N.; Verschueren, I.; Figdor, C.; Van der Meer, J.W.; Torensma, R. and Kullberg, B.J. (2004). Human dendritic cells are less potent at killing *Candida albicans* than both monocytes and macrophages. Microbes and Infection, **6**(11):985-989. doi: 10.1016/j.micinf.2004.05.013.

Netea, M.G; Brown, G.D.; Kullberg, B.J. and Gow, N.A. (2008). An integrated model of the recognition of *Candida albicans* by the innate immune system. Nature Reviews Microbiology, **6**(1):67-78. doi:10.1038/nrmicro1815.

Nguyen, H.T.T.; Park, A.R.; Hwang, I.M. and Kim, J.C. (2021). Identification and delineation of action mechanism of antifungal agents: Reveromycin E and its new derivative isolated from *Streptomyces* sp. JCK-6141. Postharvest Biology and Technology, 182, 111700. doi:10.1016/j.postharvbio.2021.111700

Niu, X.X.; Li,T.; Zhang, X.; Wang, S.X. and Liu, Z.H. (2017). *Lactobacillus crispatus* modulates vaginal epithelial cell innate response to *Candida albicans*. Chinese Medical Journal, **130**(03):273-279. doi: 10.4103/0366- 6999.198927.

Nobile, C.J.; Nett, J.E.; Hernday, A.D.; Homann, O.R.; Deneault, J.S.; Nantel, A.; Andes, D.R.; Johnson, A.D. and Mitchell, A.P. (2009). Biofilm matrix regulation by *Candida albicans* Zap1. PLoS Biology, **7**(6):e100 0133. doi: 10.1371/journal.pbio.1000133.

O'Hanlon, D.E.; Moench, T.R. and Cone, R.A. (2013). Vaginal pH and microbicidal lactic acid when *lactobacilli* dominate the microbiota. PloS one, **8**(11):e80074. doi: 10.1371/journal.pone.0080074.

Pang, H.; Qin, G; Tan, Z.; Li, Z.; Wang, Y. and Cai, Y. (2011). Natural populations of lactic acid bacteria associated with silage fermentation as determined by phenotype, 16S ribosomal RNA and recA gene analysis. Systematic and Applied Microbiology, **34**(3):235-241. doi: 10.1016/j.syapm.2010.10.003.

Paramythiotou, E.; Frantzeskaki, F.; Flevari, A.; Armaganidis, A. and Dimopoulos, G. (2014). Invasive fungal infections in the ICU: how to approach, how to treat. Molecules, **19**:1085-1119. doi: 10.3390/molecules 19011085.

Parolin, C.; Marangoni, A.; Laghi, L.; Foschi, C.; Nahui Palomino, R.A.; Calonghi, N.; Cevenini, R. and Vitali, B. (2015). Isolation of vaginal *lactobacilli* and characterization of anti-*Candida* activity. PloS one, **10**(6):e0131220. doi: 10.1371/journal.pone.0131220.

Peleg, A.Y.; Hogan, D.A. and Mylonakis, E. (2010). Medically important bacterial-fungal interactions. Nature Reviews Microbiology, **8**(5):340-349. doi: 10.1038/nrmicro2313.

Pfaller, M.A. and Diekema, D. (2007). Epidemiology of invasive candidiasis: a persistent public health problem. Clinical Microbiology Reviews, **20**(1):133-163.

Phan, Q.T.; Myers, C.L.; Fu, Y.; Sheppard, D.C.; Yeaman, M.R.; Welch, W.H.; Ibrahim, A.S.; EdwardsJr, J.E. and Filler, S.G. (2007). Als3 is a *Candida albicans* invasin that binds to cadherins and induces endocytosis by host cells. PLoS Biology, **5**(3):e64. doi: 10.1371/journal.pbio.0050064.

Pinkett, H.W.; Lee, A.T.; Lum, P.; Locher, K.P. and Rees, D.C. (2007). An inward-facing conformation of a putative metal-chelate-type ABC transporter. Science, **315**(5810):373-377. doi: 10.1126/science. 1133488.

Prasad, R. and Goffeau, A. (2012). Yeast ATP-binding cassette transporters conferring multidrug resistance. Annual Review of Microbiology, **66**:39-63. doi: 10.1146/annurev-micro-092611-150111.

Rani, A. and Azmi, W. (2019). An overview on biosynthesis and applications of extracellular pyocyanin pigment and its role in *Pseudomonas aeruginosa* pathogenesis. Ann. Phytomed., **8**:28-42. doi:10.21276/ ap.2019.8.2.4.

Ribeiro, F. de C.; Junqueira, J.C.; dos Santos, J.D.; de Barros, P.P.; Rossoni, R.D.; Shukla, S. and Mylonakis, E. (2020). Development of probiotic formulations for oral candidiasis prevention: Gellan gum as a carrier to deliver *Lactobacillus paracasei* 28.4. Antimicrobial Agents and Chemotherapy. doi:10.1128/aac.02323-19

Rivardo, F.; Turner, R.J.; Allegrone, G.; Ceri, H. and Martinotti, M.G. (2009). Anti-adhesion activity of two biosurfactants produced by *Bacillus* spp. prevents biofilm formation of human bacterial pathogens. Applied Microbiology and Biotechnology, **83**:541-553. doi:10.1007/ s00253-009-1987-7.

Robbins, N.; Uppuluri, P.; Nett, J.; Rajendran, R.; Ramage, G.; Lopez-Ribot, J.L.; Andes, D. and Cowen, L.E. (2011). Hsp90 governs dispersion and drug resistance of fungal biofilms. PLoS Pathogens, **7**(9):e1002257. doi: 10.1371/journal.ppat.1002257.

Robinson, M.J.; Osorio, F.; Rosas, M.; Freitas, R.P.; Schweighoffer, E.; Grob, O. and Reis e Sousa, C. (2009). Dectin-2 is a Syk-coupled pattern recognition receptor crucial for Th17 responses to fungal infection. Journal of Experimental Medicine, **206**(9):2037-2051. doi: 10.1084/jem. 20082818.

Rodrigues, L.; Banat, I.M.; Teixeira, J. and Oliveira, R. (2006). Biosurfactants: potential applications in medicine. Journal of Antimicrobial Chemotherapy, **57**(4):609-618. doi: 10.1093/jac/dkl024.

Sahin, Z.; Ertas, M.; Berk, B.; Biltekin, S.N.; Yurttas, L. and Demirayak, S. (2018). Studies on non-steroidal inhibitors of aromatase enzyme; 4-(aryl/heteroaryl)-2-(pyrimidin-2-yl)thiazole derivatives. Bioorganic and Medicinal Chemistry, **26**(8):1986-1995. doi:10.1016/j.bmc. 2018.02.048

Sangeorzan, J.A.; Bradley, S.F.; He, X.; Zarins, L.T.; Ridenour, G.L.; Tiballi, R.N. and Kauffman, C.A. (1994). Epidemiology of oral candidiasis in HIV-infected patients: colonization, infection, treatment, and emergence of fluconazole resistance. The American Journal of Medicine, **97**(4):339-346. doi:10.1016/0002-9343(94)90300-X.

Sobel, J.D.; Wiesenfeld, H.C.; Martens, M.; Danna, P.; Hooton, T.M.; Rompalo, A. and Chu, T.C. (2004). Maintenance fluconazole therapy for recurrent vulvovaginal candidiasis. New England Journal of Medicine, **351**(9):876-883. doi: 10.1056/NEJMoa033114.

Soloviev, D.A.; Jawhara, S. and Fonzi, W.A. (2011). Regulation of innate immune response to *Candida albicans* infections by αMβ2-Pra1p interaction. Infection and Immunity, **79**(4):1546-1558. doi:10. 1128/IAI.00650-10.

Stieglmeier, M.; Wirth, R.; Kminek, G. and Moissl-Eichinger, C. (2009). Cultivation of anaerobic and facultatively anaerobic bacteria from spacecraft-associated clean rooms. Applied and Environmental Microbiology, **75**(11):3484-3491. doi: 10.1128/AEM.02565-08.

Suissa, R.; Oved, R.; Jankelowitz, G.; Turjeman, S.; Koren, O. and Kolodkin-Gal, I. (2021). Molecular genetics for probiotic engineering: dissecting lactic acid bacteria. Trends in Microbiology., doi:10.1016/j.tim.2021.07. 007.

Sun, J.N.; Solis, N.V.; Phan, Q.T.; Bajwa, J.S.; Kashleva, H.; Thompson, A.; Liu, Y.; Dongari-Bagtzoglou, A.; Edgerton, M. and Filler, S.G. (2010). Host cell invasion and virulence mediated by *Candida albicans* Ssa1. PLoS Pathogens, **6**(11):e1001181. doi: 10.1371/journal.ppat.1001181.

Tachedjian, G.; Aldunate, M.; Bradshaw, C.S. and Cone, R.A. (2017). The role of lactic acid production by probiotic *Lactobacillus* species in vaginal health. Research in Microbiology, **168**(9-10):782-792. doi: 10.10 16/j.resmic.2017.04.001.

Taff, H.T.; Nett, J.E.; Zarnowski, R.; Ross, K.M.; Sanchez, H.; Cain, M.T.; Hamaker, J.; Mitchell, A.P. and Andes, D.R. (2012). A *Candida* biofilm-induced pathway for matrix glucan delivery: implications for drug resistance. PLoS Pathogens, **8**(8):e1002848. doi: 10.1371/journal.ppat. 1002848.

Talapko, J.; Juzbasic, M.; Matijevic, T.; Pustijanac, E.; Bekic, S.; Kotris, I. and Skrlec, I. (2021). *Candida albicans*-the virulence factors and clinical manifestations of infection. Journal of Fungi, **7**(2):79. doi: 10.3390/ jof7020079.

Tamang, B.; Tamang, J.P.; Schillinger, U.; Franz, C.M.; Gores, M. and Holzapfel, W.H. (2008). Phenotypic and genotypic identification of lactic acid bacteria isolated from ethnic fermented bamboo tender shoots of North East India. International Journal of Food Microbiology, **121**(1):35-40. doi: 10.1016/j.ijfoodmicro.2007.10.009.

Theiss, S.; Ishdorj, G.; Brenot, A.; Kretschmar, M.; Lan, C.Y.; Nichterlein, T.; Hacker, J.; Nigam, S.; Agabian, N. and Kohler, G.A. (2006). Inactivation of the phospholipase B gene PLB5 in wild-type *Candida albicans* reduces cell-associated phospholipase A2 activity and attenuates virulence. International Journal of Medical Microbiology: IJMM, **296**(6):405-420. doi: 10.1016/j.ijmm.2006.03.003.

Toth, Z.; Forgacs, L.; Kardos, T.; Kovács, R.; Locke, J. B.; Kardos, G. and Majoros, L. (2020). Relative Frequency of Paradoxical Growth and Trailing Effect with Caspofungin, Micafungin, Anidulafungin, and the Novel Echinocandin Rezafungin against Candida Species. Journal of Fungi, 6(3):136. doi:10.3390/jof6030136

Vaishali; Khan, J.and Azmi, W. (2021). Strategies for the management of *Candida albicans* infection. Ann. Phytomed., **10**(2):145-156. doi: 10.21276/ap.2021.10.2.20.

Van Daele, R.; Spriet, I.; Wauters, J.; Maertens, J.; Mercier, T.; Van Hecke, S. and Brüggemann, R. (2019). Antifungal drugs: What brings the future? Medical Mycology, **57**(Supplement_3):S328-S343. doi:10.1093/ mmy/myz012

van Hoogmoed, C.G.; van der Mei, H.C. and Busscher, H.J. (2004). The influence of biosurfactants released by *S. mitis* BMS on the adhesion of pioneer strains and cariogenic bacteria. Biofouling, **20**(6):261-267. doi:10.1080/08927010400027050.

van Reenen, C.A. and Dicks, L.M. (2011). Horizontal gene transfer amongst probiotic lactic acid bacteria and other intestinal microbiota: what are the possibilities? A review. Archives of Microbiology, **193**:157-168. doi:10.1007/s00203-010-0668-3.

Vitiello, A.; Ferrara, F.; Boccellino, M.; Ponzo, A.; Cimmino, C.; Comberiati, E.; Zovi, A.; Clemente, S. and Sabbatucci, M. (2023). Antifungal Drug Resistance: An Emergent Health Threat. Biomedicines, **11**(4):1063. doi: 10.3390/biomedicines11041063.

Vylkova, S.; Carman, A.J.; Danhof, H.A.; Collette, J.R.; Zhou, H. and Lorenz, M.C. (2011). The Fungal Pathogen *Candida albicans* Autoinduces Hyphal Morphogenesis by Raising Extracellular pH. mBio, **2**(3). doi:10.1128/mbio.00055-11.

Wachtler, B.; Wilson, D.; Haedicke, K.; Dalle, F. and Hube, B. (2011). From attachment to damage: defined genes of *Candida albicans* mediate adhesion, invasion and damage during interaction with oral epithelial cells. PloS One, **6**(2): e17046. doi: 10.1371/journal.pone.0017046.

Wachtler, B.; Citiulo, F.; Jablonowski, N.; Forster, S.; Dalle, F.; Schaller, M.; Wilson, D. and Hube, B. (2012). *Candida albicans*-epithelial interactions: dissecting the roles of active penetration, induced endocytosis and host factors on the Infection Process. PloS one, **7**(5): e36952. doi: 10.1371/journal.pone.0036952.

Wagner, R.D.; Pierson, C.; Warner, T.; Dohnalek, M.; Hilty, M. and Balish, E. (2000). Probiotic Effects of Feeding Heat-Killed *Lactobacillus acidophilus* and *Lactobacillus casei* to *Candida albicans*-Colonized Immunodeficient Mice. Journal of Food Protection, **63**(5):638-644. doi:10.4315/0362-028x-63.5.638

Weindl, G.; Naglik, J.R.; Kaesler, S.; Biedermann, T.; Hube, B.; Korting, H.C. and Schaller, M. (2007). Human epithelial cells establish direct antifungal defense through TLR4-mediated signaling. The Journal of Clinical Investigation, **117**(12):3664-3672. doi: 10.1172/JCI28115.

Weissman, Z. and Kornitzer, D. (2004). A family of *Candida* cell surface haem-binding proteins involved in haemin and haemoglobin-iron utilization. Molecular Microbiology, **53**(4):1209-1220. doi:10.1111/j.1365-2958.2004. 04199.x.

Wells, C.A.; Salvage-Jones, J.A.; Li, X.; Hitchens, K.; Butcher, S.; Murray, R.Z. and Ashman, R.B. (2008). The macrophage-inducible C-type lectin, mincle, is an essential component of the innate immune response to *Candida albicans*. The Journal of Immunology, **180**(11):7404-7413. doi: 10.4049/jimmunol.180.11.7404.

Wisplinghoff, H.; Bischoff, T.; Tallent, S.M.; Seifert, H.; Wenzel, R.P. and Edmond, M.B. (2004). Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clinical Infectious Diseases, **39**(3):309-317. doi:10.1086/421946.

Wu, A.; Grela, E., Wójtowicz, K.; Filipczak, N.; Hamon, Y.; Luchowski, R. and Trombik, T. (2019). ABCA1 transporter reduces amphotericin B cytotoxicity in mammalian cells. Cellular and Molecular Life Sciences, doi:10.1007/s00018-019-03154-w

Xu, J.; Cao, Y.; Zhang, J.; Yu, S.; Zou, Y.; Chai, X.; Wu, Q.; Zhang, D.; Jiang, Y. and Sun, Q. (2011). Design, synthesis and antifungal activities of novel 1, 2, 4-triazole derivatives. European Journal of Medicinal Chemistry, **46**(7):3142-3148. doi: 10.1016/j.ejmech.2011.02.042.

Zangl, I.; Pap, I.J.; Aspock, C. and Schuller, C. (2020). The role of *Lactobacillus* species in the control of *Candida via* biotrophic interactions. Microbial Cell, **7**(1):1-14. doi: 10.15698/mic2020.01.702.

Zeise, K.D.; Woods, R.J. And Huffnagle, G.B. (2021). Interplay between *Candida albicans* and lactic acid bacteria in the gastrointestinal tract: impact on colonization resistance, microbial carriage, opportunistic infection, and host immunity. Clinical Microbiology Reviews, **34**(4):e00323-20. doi: 10.1128/CMR.00323-20.

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